Figure 1: Extrapolated response data of amino acid concentration (2x concentration) versus yeast extract concentration (g/L) at iron concentration of 0.34 mL/L

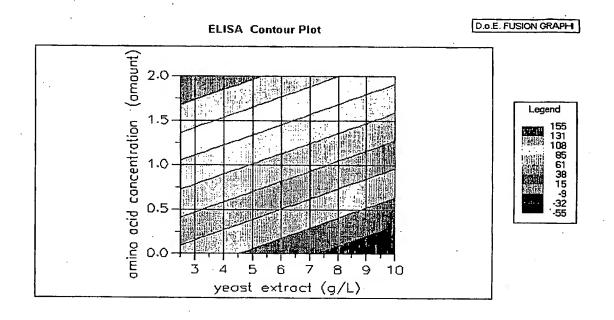
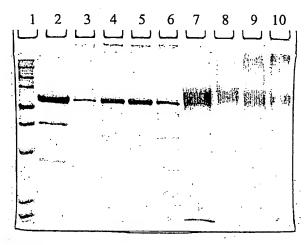


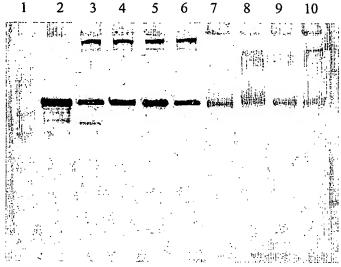
Figure 2 SDS-PAGE analysis of diphtheria toxin and toxoid produced using the animal-component containing and animal-component free media;



## Well#

- 1. BioRad MW markers
- 2. Diphtheria Toxin CO3105
- 3. Diphtheria Toxin Diph-20L-40F (Animal Component Containing Medium)
- 4. Diphtheria Toxin Diph-20L-48F (CDM + Yeast Extract Containing Medium)
- 5. Diphtheria Toxin Diph-20L-50F (CDM + Yeast Extract Containing Medium)
- 6. Diphtheria Toxin Diph-20L-55F (CDM + Yeast Extract Containing Medium)
- 7. Diphtheria Toxoid CO3152
- 8. Diphtheria Toxoid Diph-20L-40F(Animal Component Containing Medium)
- 9. Diphtheria Toxoid Diph-20L-48F(CDM + Yeast Extract Containing Medium)
- 10. Diphtheria Toxoid Diph-20L-50F(CDM + Yeast Extract Containing Medium

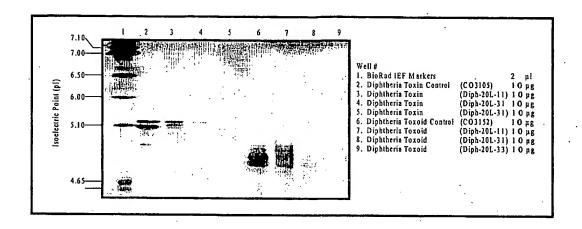
Figure 3. Western Blot analysis of diphtheria toxin and toxoid produced using the animal-component containing and animal-component free media.



## Well#

- 1. BioRad MW markers
- 2. Diphtheria Toxin CO3105
- 3. Diphtheria Toxin Diph-20L-40F (Animal Component Containing Medium)
- 4. Diphtheria Toxin Diph-20L-48F (CDM + Yeast Extract Containing Medium)
- 5. Diphtheria Toxin Diph-20L-50F (CDM + Yeast Extract Containing Medium)
- 6. Diphtheria Toxin Diph-20L-55F (CDM + Yeast Extract Containing Medium)
- 7. Diphtheria Toxoid CO3152
- 8. Diphtheria Toxoid Diph-20L-40F(Animal Component Containing Medium)
- 9. Diphtheria Toxoid Diph-20L-48F (CDM + Yeast Extract Containing Medium)
- 10. Diphtheria Toxoid Diph-20L-50F(CDM + Yeast Extract Containing Medium)

Figure 4. Isoelectric gel analysis of diphtheria toxin and toxoid produced using the animal-component containing and animal-component free media.



- 1. BioRad IEF markers
- 2. Diphtheria Toxin CO3105 (Animal Component Containing Medium)
- 3. Diphtheria Toxin Diph-20L-11 (NZ Amine Containing Medium)
- 4. Diphtheria Toxin Diph-20L-31 (CDM + Yeast Extract Containing Medium)
- 5. Diphtheria Toxin Diph-20L-31 (CDM + Yeast Extract Containing Medium)
- 6. Diphtheria Toxoid CO3152(Animal Component Containing Medium)
- 7. Diphtheria Toxoid Diph-20L-11(CDM + Yeast Extract Containing Medium)
- 8. Diphtheria Toxoid Diph-20L-31 (CDM + Yeast Extract Containing Medium)
- 9. Diphtheria Toxoid Diph-20L-31(CDM + Yeast Extract Containing Medium)

Figure 5. Circular dichroism of diphtheria toxin produced using the animal-component containing and animal-component free media.

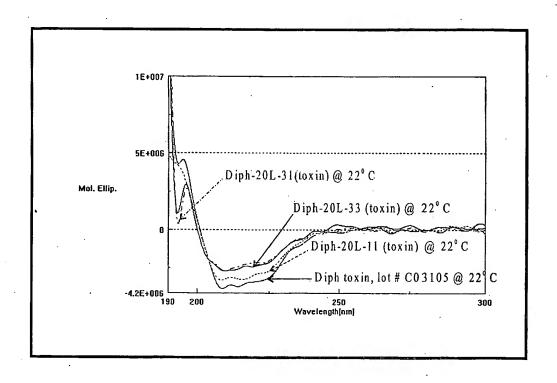


Figure 6. Circular dichroism of diphtheria toxoid produced using the animal-component containing and animal-component free media.

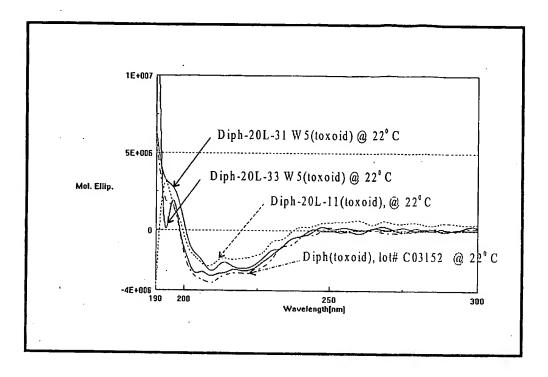


Figure 7: Circular Dichroism Analysis of Diphtheria Toxin and Toxoid for Diph 200L-82 (similar batch to Diph-20L-64 but performed at 200 L scale)

